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DT01 Rec'd PCT/PTO 20 DEC 2004**BIOLOGICAL REACTION APPARATUS WITH DRAINING MECHANISM****Field of the Invention**

5 The present invention relates to a method or apparatus for providing a reaction chamber for chemical reactions. The present invention also relates to a method of filling a reaction chamber and a fluid used for this purpose.

**Background of the invention**

10 There are many applications where it is desirable to initiate a chemical reaction on a sample. Commonly the samples are located on a microscope slide. Typical reactions include immuno-histochemical reactions of cellular material, or in situ-hybridisation of DNA or RNA. In other forms, microarrays of thousands of small samples of material, including DNA, RNA proteins or small chemical compounds are attached to a microscope slide, where it is desirable to promote a chemical reaction between the material on the slide and other chemicals or fluids. These reactions require controlled  
15 conditions, including controlled reaction time, temperature and concentration of chemicals. It is important that the reaction across the slide is uniform, and also that reactions from slide to slide are consistent.

It is also important to minimise evaporation and overall fluid quantity used.

20 In the past, chemical reactions taking place on slides have been controlled by skilled persons adding and mixing the reagents. This allowed the time and quantity of the reagents to be controlled for each slide. However, this procedure is time consuming, required highly skilled operators, and can produce inconsistent results from slide to slide.

**Summary of the Invention**

25 In one form, the present invention is a biological reaction apparatus for receiving at least one substrate having a sample located in a sample region, and a separate cover, such that a reaction chamber is formed between the cover and substrate over the sample region, wherein the apparatus includes

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a locating means to locate the substrate;

a cover locating means for locating and moving the cover with respect to the substrate;

a fluid dispensing means for dispensing fluid into the reaction chamber; and

5 a draining mechanism;

wherein the draining mechanism includes wicking means.

Preferably the wicking means include points of contact on the substrate to provide a fluid path to drain fluid from the substrate.

10 Preferably the substrates are supported in the apparatus from underneath. Supporting substrates from underneath removes wicking paths from around the periphery of the substrate, which reduces fluid usage and loss.

In another form, the present invention provides a fill fluid for performing a filling of a reaction chamber, where the fill fluid has a viscosity higher than an antecedent fluid on a substrate.

15 Preferably the fill fluid is miscible with water

Preferably the fill fluid has a higher boiling point than water.

Preferably the fill fluid leaves no residue on the substrate or sample.

Preferably the fill fluid is inert to biological reagents and samples.

Preferably the fill fluid is a solution comprising glycerol.

20 In one form the fill fluid contains glycerol, water, and buffer. The buffer may be tris buffered saline.

Preferably the fill fluid contains between 2% to 80% glycerol by volume.

More preferably still the fill fluid contains between 10%-60% glycerol per volume.

More preferably the fill fluid contains between 20% to 30% glycerol.

25 In one form the fill fluid includes a surfactant to aid in the disbursement of any bubbles formed within the reaction chamber during a fill cycle.

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More preferably the surfactant is Tween.

In another form the present invention relates to a receptacle for substrates having receiving means adapted to locate a substrate and a cover.

5 Preferably the receiving means includes stations to locate and support the substrate, and the cover is supported on the substrate.

Preferably the receiving stations support the substrate around part of a periphery of the substrate.

Preferably the receiving means are defined by a respective aperture having peripheral ledges for supporting the substrates.

10 Preferably the apertures are adapted to receive support platforms from a reaction apparatus, such that when loaded in a reaction apparatus, the platforms support the substrates.

Preferably the receiving means have a lifting means for lifting the covers from the substrate.

15 More preferably the lifting means are ramps adapted to engage with projections on the cover.

Preferably the receiving means have guides allowing the cover to be moved with respect to the receptacle and slide.

20 In another form the present invention relates to a dispenser for a reaction apparatus including a fluid conduit,

a pump connected to the fluid conduit;

a locating means for moving the fluid conduit from a fluid source to a dispensing region.

25 Preferably the dispenser includes a bar code sensor to detect the type of fluid source and substrate;

Preferably the dispenser includes a means for determining the volume of fluid remaining in a fluid source.

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More preferably the means for determining the volume of fluid in a fluid source includes a sensor adapted to measure the level of fluid in a fluid container.

More preferably the sensor measures a change of capacitance of the fluid conduit to detect insertion into a fluid in the fluid container.

- 5 In another form the present invention relates to a method of dispensing fluid to a substrate including the steps of:

loading a reagent receptacle with at least one fluid container;

mounting the reagent receptacle to a reaction apparatus

detecting the reagent receptacle

- 10 once the reagent receptacle is detected, initiating a sensor to detect the type of fluid within the at least one fluid container

storing the information on fluid type to allow the fluid to be dispensed onto a substrate when required.

Preferably the sensor detects bar codes.

- 15 In another form the present invention relates to a reaction apparatus having a support projection for a slide, a dispensing means and a fluid removal means, where the support projection is adapted to support a slide from underneath, and a wicking means contacting the periphery of the slide, such that the wicking means provides a wicking path to remove fluid from the upper surface of the slide.

- 20 Preferably the support projection is angled between 0 and 10 degrees to the horizontal providing the apparatus with a fluid removal region. This provides a gradient to promote fluid flow.

Preferably the wicking means is wicking posts.

Preferably the wicking posts are located at the fluid removal region.

- 25 In one form the wicking means is adapted to extend across a significant proportion of the width of the substrate.

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In another form the present invention relates to a reaction apparatus adapted to locate a substrate having a surface containing a sample and cover having a surface forming a reaction chamber with the sample containing surface, including a cover engaging means adapted to change the volume of the reaction chamber.

- 5 This promotes mixing of fluid within the reaction chamber.

In one form the cover engaging means is a clamping mechanism adapted to clamp the cover to the substrate.

In another form the present invention relates to a reaction apparatus having a separate substrate tray:

- 10 the substrate tray adapted to hold a number of substrates and covers;  
at least one receiving station for receiving said substrate tray;  
a dispensing means for dispensing fluid onto substrates in the substrate tray  
wherein a reaction chamber is formed between the substrate and cover, such that fluid dispensed onto the substrates enters the reaction chamber.
- 15 Preferably the reaction apparatus has a number of receiving stations, each station adapted to receive a substrate tray.
- Preferably the reaction apparatus has a controller which allows the fluid to be dispensed onto a substrate on one substrate tray independently of any other substrate tray.
- 20 In another aspect, there is provided reaction apparatus for receiving a substrate having a sample located in a sample region and a draining mechanism including wicking means for draining fluid from the substrate.

In another aspect, there is provided a method of forming a reaction chamber on a slide in a reaction apparatus including:

- 25 placing a cover having a cavity on a slide, forming a reaction chamber;  
locating the cover and slide in a receptacle of a tray;

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providing a receiving portion in the reaction apparatus having a mount for each receptacle in the tray;

loading the tray into a receiving portion of the reaction apparatus, where the receiving portion of the reaction apparatus locates the tray;

- 5    releasably holding the cover to the slide; and  
releasing the tray from the slide and cover.

In another aspect, there is provided an apparatus for loading multiple slides and covers including:

- a tray having a number of receptacles for slides and covers;
- 10    a receiving portion for receiving trays;  
mounts for each receptacle located in the receiving portions;  
a clamp for each mount;
- wherein when a tray having slides and covers is loaded into the receiving portion, each clamp holds the cover on the slide to locate the slide, and the tray drops from the
- 15    slides so each slide is supported by the mount.

In another aspect, there is provided a method of undertaking reactions on samples on slides involving multiple steps including:

- loading a first holder having at least one slide into a reaction apparatus;
- scanning the slide to determine the multiple steps in the reaction to take place on the
- 20    slide;
- determining whether other holders have been loaded into the reaction apparatus;
- undertaking the multiple steps required on the at least one slide associated with the first holder;
- when a second holder is detected, continue the steps in the reaction associated with
- 25    the at least one slides in the first holder and then undertaking the at least one steps associated with the slides associated with the second holder.

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In another aspect, there is provided an apparatus for performing reactions on slides including:

a tray having a plurality of receptacles adapted to support and locate slides and associated covers;

5 receiving ports for the trays, the receiving ports having mounts associated with each receptacle of the tray;

a clamping mechanism for clamping the cover and slide in place;

a fluid draining means for draining fluid from the reaction chamber formed between the cover and slide;

10 fluid receptacles to allow at least one fluid to be placed on the apparatus;

fluid dispensing means to dispense fluid onto the slides;

wherein once the tray is loaded, the slides and cover are clamped and the tray is moved so that the slides and covers are supported on the mounts, fluid may be dispensed onto the slides by the dispensing means, and drained by the draining means.

15 In another aspect, there is provided an apparatus for applying reagents to sample slides, including:

a plurality of ports for receiving the slides;

a reader for reading identification information on each of the slides; and

a reagent rack for receiving reagent containers which carry reagent to be deposited on

20 the slides; wherein

the slides are provided on trays, which are received in the associated ports such that each tray represents a separate batch of slides, to allow for addition and removal of separate trays, for batch processing during operation of the apparatus.

#### **Brief Description of the Drawings**

25 The invention is described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

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Figure 1 shows an example of a reaction apparatus;

Figure 2 shows an example of a tray used with the reaction apparatus of figure 1;

Figure 3 shows the tray of figure 2 partially loaded into a receiving port of the reaction apparatus of figure 1;

- 5 Figure 4 shows an example of a reagent container rack and rack receiving zone of the reaction apparatus;

Figure 5 shows a robotic arm and dispensing mechanism of the reaction apparatus of figure 1;

- Figure 6 shows slides and covers loaded onto stations of a reaction apparatus of figure 1;  
10 1;

Figure 7 shows a cover loaded into a tray shown in figure 2;

Figure 8(a) – (c) shows a cover in three positions relative to a slide;

Figure 9 shows a first view of an engaging means for a cover in a receiving port of the reaction apparatus of figure 1;

- 15 Figures 10 shows a schematic section of a reaction chamber formed between a cover and a slide;

Figure 11 shows a washing station for the reaction apparatus;

Figure 12 shows a station of a tray receiving port and wicking means.

Figure 13 shows a cut away section of a cover mounted upon a slide;

- 20 Figure 14 shows a top view of a tray receiving port of the reaction apparatus of figure 1;

Figure 15 shows a cross section of a slide and cover on a mount of a station;

Figure 16 shows a cutaway view of sections of the slide, cover, and mount of figure 15; and

- 25 Figure 17 is a perspective view of a mixing station.

#### **Detailed Description**



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Figure 1 shows an automated reaction apparatus 10 having bulk reagent container receiving zone 12, substrate tray receiving ports 14, a robotic arm 16 and a reagent rack receiving zone 18.

5 Bulk container receiving zone 12 is adapted to hold a number of bulk reagent containers 20. These containers 20 typically hold fluids such as tris buffered saline, PBS, Citrate, EDTA, organic solvents, waste reagents, deionised water, and dewaxing solutions. The bulk reagent containers of the apparatus 10 hold 1 to 4 litres of fluid.

10 The robotic arm 16 is moveable along the guide 24, driven by motors (not shown) and controlled by a controller (not shown) such as a computer. As shown in figure 5 a dispensing means 26 is moveably mounted to arm 16, and includes a fluid conduit such as pipette 28; for dispensing fluids. The pipette 28 is attached by tubing 29 to a pump (not shown) which in this example is a motorised syringe pump capable of withdrawing, holding and delivering an accurate volume of fluid. The pipette 28 may be lowered when withdrawing or dispensing fluids, and raised when moving across  
15 the apparatus 10. A sensor 33 for reading bar codes is also included on the arm 16.

The reagent rack receiving zone 18 includes 4 rack mounts 30, rack locating clip 31 and a sensor 35 for detecting the mounting of each reagent rack 34, as best seen in figure 4. The reagent racks 34 each includes nine receptacles 36, each adapted to receive a reagent container 39. The reagent racks 34 may be removed from the rack  
20 receiving zone 18 when it is necessary to remove, refill or change a container 39.

In figures 1 and 3 there are three slide tray receiving ports 14 and each is adapted to hold a single slide tray 15.

25 The slide tray 15 (shown in figure 2) includes ten slide receiving means 37, in the form of apertures which have support means 38. One or more substrates in the form of slides 1 may be placed into the slide tray 15, as shown in figure 3, such that the slides 1 are supported around the periphery but not in the middle. Covers 2 are placed onto the slides 1 as shown in figure 7. When the slide tray 15 is placed into the tray receiving port 14, each receiving means 37 corresponds to a slide station 35a in the apparatus 10 as shown in figure 6 and described in further detail below. A series of

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blocks 40 in the tray receiving ports 14 are adapted to support the slides 1 when the slide tray 15 is fully inserted into the apparatus 10 along rails 39a. When the slide tray 15 is inserted fully into the receiving port 14, it may be lowered such that the slides come into contact with and are supported by the blocks 40. The slide tray 15 is then not in contact with the slides, leaving the slides supported from underneath by the blocks 40. While only two slides 1 and covers 2 are shown loaded onto the tray 15 shown in figure 3, there may be any number of slides and covers, up to the number of receiving means 37 contained by slide tray 15.

The blocks 40, which are typically metal and may be controllably heated or cooled, support the slides 1 in conjunction with wicking means 41 in the form of wicking posts 42 as shown in figure 12. The upper surface of blocks 40 are inclined at a small angle to the horizontal (typically 5 degrees) to promote fluid flow along the slide during operation of the apparatus 10.

The cover 2 (best seen in figures 8 and 13) is one of a number of variations possible, other variations being described in copending International patent application titled "A cover for a slide" by the same applicant and hereby incorporated by reference. The cover 2 is made from a clear plastic material, and is substantially the same width as the slide 1 to which it is to be mounted. A cavity 51 is located on side a of the cover 2 that faces the sample, and this cavity 51 in conjunction with lands 52 and sample holding surface 53 of the slide forms a reaction chamber 32 as shown in schematic figure 10, where the z axis has been exaggerated for clarity. Figure 10 is a sectioned view of a cover over a slide 1 showing the reaction chamber 32, sample 5, lands 52 and slide surface 53. Typically the slide is 25mm wide by 76mm long, and the cavity is 100 micrometres high. The land 52 is in close proximity to or contacts slide surface 53 along contact surface 54 as shown in figure 13, and therefore restricts fluid leakage from the reaction chamber 32 outside the reaction chamber. Capillary forces assist in holding the fluid in the reaction chamber 32.

A locator arm 3 enables the cover 2 to be moved along the slide 1 by a locator engaging means 43 shown in figure 9. Each locator arm 3 is engaged by a bracket 44. A range of positions of the cover relative to the slides is shown in figure 8, where

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figure 8 (a) is fully open, figure 8(b) is partially open and figure 8(c) is fully closed. A reaction chamber 32 is formed between the cover 2 and slide 1 over a sample 5 on the slide 1 when the cover is in a closed or partially open position. The cover 2 includes a fluid reservoir 19 where fluid may be dispensed. There are several forms  
5 of fluid reservoir, as described in the abovementioned copending application. The cover and slide are capable of holding fluid in the reservoir 19, when the cover is in contact with the slide.

The fluid in the reservoir is drawn into the cavity 51 of the cover as the cover moves over the slide from an open position shown in figure 8(a) to a closed position shown  
10 in figure 8(c). The reservoir 19 may hold sufficient volume such that there is still fluid in the reservoir when the cover is in a closed position, and this provides a reservoir of fluid to reduce the need for fluid top ups during extended reaction times or sustained high temperatures. It is believed that the fluid is drawn into the cavity by a number of factors including capillary forces.

15 The covers 2 include wings 50 projecting from cover 2 adapted to engage ramps 52 on the slide tray 15, as shown in figure 7. The wings lift the cover 2 clear from the slide 1 when the wings 50 on the cover 2 engage lifting means in the form of ramps 52. It is possible to move the cover 2 to a position where the sample is uncovered but the cover remains in contact with the slide, along guides 56. Depending on the  
20 configuration of the ramps 52 and wings 50, it may not be necessary to completely open the chamber before the cover loses contact with the slide 1.

The arm 3 is moved by an actuator such as a cam arrangement (not shown) which engages positioning member 45 controllably so that the cover is able to be accurately positioned with respect to the slide along the x-axis shown in figure 8. While figure 9  
25 shows that all covers are moved at once, in other examples of reaction apparatus it is possible to have individual control of the covers by moving arms individually.

In figure 6, slides 1 having bar codes 6 are shown on their respective blocks 40. For the purposes of this diagram the slide tray 15 and engaging means 43 have been omitted from view for clarity. A clamp 60 is used to hold a cover 2 securely in  
30 position on the slide 1 during a processing step. Clamp 60 includes a number of legs

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62, which are situated around the periphery of the slide 1 and have spring like properties to provide an even force around the periphery of the cover. The clamp 60 may be made from a plastic material, and in another example (not shown) the legs may be made from metal, in the form of a spring (leaf or coil). Other forms of legs or  
5 clamp are possible such as compressible foam or pneumatic clamps.

The clamp 60 for each cover 2 may be raised when the cover 2 is to be moved, or lowered to engage the cover 2 during a fluid dispensing operation. In the present example, all clamps 60 and covers 2 in a particular receiving port 14 are moved together. Individual receiving ports 14 may operate independently of each other.

10 In use, bulk reagents in bulk reagent containers 20 are loaded into the apparatus 10. Reagent racks 34 having reagent containers 39 are loaded into the rack mounts 30. Sensors 35 detect their presence and the bar code sensor 33 reads the bar codes on each reagent container 39 to identify the contents of each reagent container 39 relative to its position in the reagent rack 34. Information relating bar codes 6 on slides 1 to  
15 samples on the slides 1 and bar codes 6 on reagent containers 39 relating to their respective contents, is input into the controller (not shown), which is typically a computer work station having an appropriate software interface and drivers. A slide tray 15 containing at least one slide 1, but up to ten slides, is placed into the receiving port 14, whereupon a sensor (not shown) detects the slide tray 15 and initiates a scan  
20 of the stations 35a. When scanning, the bar code sensor 33 on the robotic arm 16 moves to each station 35a and attempts to read a bar code 6. If a slide 1 with a bar code 6 is present, the controller compares the bar code 6 with a list of known slides and information input by the user to determine which protocol to apply to each individual slide 1. Alternatively, once the bar codes have been scanned, the user  
25 inputs information required for the apparatus to process the slide. Each slide may have a different protocol. The controller compares the reagents required to perform the reactions dictated by the protocols with the reagents located in the containers 39 in the reagent racks 34. Any discrepancy will cause an error message to be sent to the user. If a reagent container 39 is missing then the reagent rack 34 may be removed

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and the correct container 39 placed in the rack 34, whereupon the rack 34 is detected and another scan of reagent containers 39 is undertaken.

If no errors are present, the robotic arm 16 moves the pipette 28 of the dispensing means 26 to the appropriate reagent container 39 and withdraws the required amount of fluid. At this time the dispensing means 26 checks the capacitance of the pipette 28, which changes when the pipette comes into contact with the fluid surface of a reagent container 39. In this way the volume of fluid remaining in the reagent container 39 can be determined and the user can replace the container 39 as necessary. The robotic arm then moves the pipette 28 to a first slide 1 (determined by the controller) and dispenses the fluid onto the surface of the slide 1. There are several options in placement of the pipette 28 and cover 2 in relation to the sample 5 on the slide 1, and these will be discussed further below.

Once the dispensing operation for a first slide 1 has been undertaken, the process is repeated for further slides. It is not necessary for each slide 1 to be filled with the same fluid at each step, and the slides may be filled in any order that is appropriate. A washing station 120 shown in figure 11 is located near the reagent racks 34 and may be used to clean the pipette 28 prior to withdrawal of a different reagent. Washing station 120 includes a receptacle 121 for receiving the pipette 28, where cleaning fluid from one of the bulk reagent containers 20 is pumped onto the outside of the pipette 28 to remove traces of the previous fluid. Cleaning fluid may also be pumped from the bulk reagent container 20 via tubing to clean the inside surfaces of the pipette 28.

Reagents may be pumped from the bulk reagent containers 20 through piping and valves (not shown) into the pipette 28. Bulk reagent from the bulk reagent containers 20 may also be pumped to a wash station 120.

Other reagent containers such as the bulk reagent containers 20, included in the body 12 of the apparatus 10, can add to the type of reagents that may be dispensed onto the slide. Some bulk reagent containers 20 normally contain fluids required for washing and hydrating samples.

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The reagent rack 34 may be used to contain a detection kit. A detection kit consists of a number of reagents in separate reagent containers 39 that are used to perform a particular test on one or more samples. Such a detection kit may include nine reagent containers 39 to perform a single test, and this reduces the number of reagent  
5 containers 39 available to other slides to twenty seven.

Typical reagents applied to samples on slides include primary antibodies, such as those sold by Novocastra Laboratories Ltd. These reagents are normally supplied in the reagent containers 39 in volumes typically between 7ml and 30ml. Other reagents and fluids, such as buffers and de-ionised water, may be kept in the bulk storage  
10 containers 20 which typically have volumes between 1-4 litres.

Some reagents, once prepared for application to a sample, have a relatively short shelf life. Therefore, either the reagent is supplied pre-mixed in a ready-to-use formulation, whereupon it must be used within a short period of time from ordering, or it may be prepared by laboratory staff prior to use, and placed into an appropriate reagent  
15 container. Some of the reagents, such as 3', 3 - diamino benzidine (DAB), when in a final form, begin to degrade soon after preparing and may not be useable more than 24 hours after initial preparation. This requires a new batch to be prepared every day, and ensuring that old batches are discarded after use. Further, enzymes such as  
20 protease may need to be applied in varying concentrations depending on factors such as tissue type, other reagents to be applied etc. This can result in numerous batches of reagents being required to be prepared before application to the samples, with the associated problems such as correct application, expiry date, correct mixing, tracking and traceability.

Concentrated primary antibodies may also require preparation before use, requiring  
25 dilution before application to a sample. Primary antibodies can be supplied either in a concentrated form or pre-diluted ready-to-use. However, it may be necessary to have several different working dilutions of the same antibody on a single apparatus 10, which would otherwise take up several locations in the reagent rack 34. It is therefore advantageous to have a single reagent container 39 of an antibody, where diluting of  
30 the antibody reagent may take place before the reagent is applied to the sample. The

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primary antibody may be diluted by a primary antibody diluent such as ABDIL 9352 sold by Vision BioSystems Ltd.

In the present embodiment of the apparatus 10, a mixing station 122 is provided, as shown in figure 11. Mixing station 122 includes an insert 130, as shown in Figure 17, having a number of mixing vials 132. The insert 130 has six vials, each vial able to hold a different reagent. The vials 132 are shown all the same volume, but may vary in volume according to requirements. Typical volumes may be 7 ml per vial.

Also mounted to the insert 130 is a tab 134. Tab 134 may be used to identify the insert 130 such as by way of a barcode. It is envisaged that as the insert 130 is disposable, but may contain a number of different reagents over the course of several runs of the apparatus 10.

The bar code on the insert 130 may be used to identify the insert 130 so that the controller knows when to discard the insert 130, and request that a new insert be loaded into the mixing station 122. This may be predetermined after a set period of time or uses.

Also shown on insert 130 is an overflow aperture 135, which is adapted to allow excess fluid to drain from the insert should any of the vials 132 overflow.

In use, information from the slide bar codes may be cross-checked with a database in the controller to establish which series of reagents is to be applied to each slide. The apparatus 10 then compares the reagents required, to the reagents currently loaded. If a reagent is identified that is not in final form for application to a sample, then a preparing step is scheduled into the order of tasks to be undertaken on the apparatus 10.

In one example, three reagent containers (identical to reagent container 39 located in the reagent rack 34) each have a component part A, B, and C of DAB may be located on the apparatus 10. In the present example DAB will be mixed in a ratio of 1 part A to 25 part B to 1 part C. To mix a batch of DAB ready for use, the robotic arm 16 first moves to the reagent container containing part A, and withdraws a set volume of part A of the reagent. The robotic arm 16 then moves to one of the vials 132 at the mixing

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- station 122 and deposits the volume into one of the vials 132. The pipette 28 then moves to a washing station 120 located next to the mixing station 122, where the outside and inside of the pipette 28 are rinsed. Once cleaned, the robotic arm 16 moves the pipette 28 to the reagent container containing part B of the reagent. The pipette 28 withdraws the reagent (25 times the volume of part A) and moves to the vial containing part A. Once deposited in the vial, the pipette 28 moves to the washing station and is again washed, before moving to the reagent container holding part C of the reagent. The same volume as removed from the container holding part A is removed, and the pipette 28 moves to the original vial and deposits the reagent with the other reagents. Initially depositing the reagents into the mixing vials causes some mixing, however additional mixing can be accomplished by withdrawing some or all of the reagent from the vial 132 into the pipette 28, then re-depositing the reagent into the vial 132. The pipette 28 may move vertically to ensure that the tip is above the fluid level when depositing to aid the mixing process. The energy of re-deposition causes the reagents to mix more readily. This mixing process can be undertaken a number of times as desired. After the reagent has been mixed sufficiently, the pipette 28 may proceed to the wash station 120 if the next reagent to be applied to a sample is not DAB. This volume of the vials and the amount withdrawn by the pipette 28 provide a sufficient volume of DAB for many applications to samples. Whenever DAB is required, the robotic arm 16 moves the pipette 28 to the vial where the DAB was mixed, as the vial in which mixing of particular reagents is recorded by the controller. The time of the preparation is also recorded, so that after a predetermined period of time the mixed reagent can be discarded. This prevents the prepared reagent from being used after expiring.
- After completion of testing for the day, or at the expiry of the DAB, the vial 132 containing the DAB (or any other reagent that has expired) can be cleaned as discussed below.

In relation to scheduling of mixing within a batch, specific details of scheduling are disclosed in Australian Provisional Patent application titled "Method of Scheduling"



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filed 24 February 2003 by same applicant, the contents of which are hereby incorporated by reference.

While the above process is automated, the resources employed (robotic arm 16 and pipette 28) may be utilised for significant periods of time in general reagent application to samples, and therefore it may be desirable to reduce the necessity to prepare several batches of reagent during a day. For this reason the apparatus 10 can be programmed to prepare reagents in the absence of any samples loaded into the apparatus 10 or during normal processing, and the volume and concentrations are user determinable through a user interface (not shown).

10 In the above example the concentration and time of preparation of each reagent in each vial 132 are stored in the memory of the controller of the apparatus 10, so there is no chance of old or incorrect mixed reagent being applied to a sample, reducing operator error.

The mixing by the pipette 28 ensures that the prepared reagent is fully mixed before application to a sample, and provides a better uniformity of mixing than, for example, applying components of the reagent directly to the sample and mixing on the sample.

Other examples of reagents that benefit from mixing on the apparatus 10 include protease, which may be required to be applied in a number of concentrations. In the above example, only one reagent container of protease would be required, and several concentrations of protease may be prepared by the apparatus 10 using diluent stored on board either in a reagent container 39 or bulk reagent container 20. These different concentrations may be placed in different vials 132 for later use.

In the above example, it is possible to have the mixing tasks scheduled into the steps of applying reagent to the samples. For example, there are often periods of time during a testing of a slide where there are no tasks required of the robot arm. These times may be referred to as open times, which typically occur when the fluid applied to a slide requires time to react before the next step is undertaken. If an open time is of a sufficient length, it may be possible to schedule in a mixing step. This minimises

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the time required to complete the application of fluid to samples, while freeing the operator from preparing the reagents.

After reagent is prepared, and it is applied to samples, remaining or expired prepared reagent is siphoned to waste by the aspirator. The vials 132 may then be cleaned.

5 Cleaning is undertaken by draining any prepared reagent remaining after the required prepared reagent has been dispensed. Draining is done with the pipette 28, the drained fluid being directed to an internally plumbed bulk waste container. Once substantially empty, a rinse cycle is undertaken. The rinse cycle may use a cleaning solution, which for example could contain an alcohol such as IMS dispensed into the  
10 vial 132. The cleaning solution is then drained via the pipette 28. More than one rinse cycle may be undertaken. After removing cleaning solution for the final rinse, any remaining cleaning solution is allowed to evaporate to completely empty the vial.

It is also possible to revisit the mixing vial after a predetermined time from initial preparation, to re-mix the reagent. This may be done by withdrawing some of the  
15 prepared reagent into the pipette 28, and redispensing into the same vial 132. This may be important where components of the prepared reagent settle after time or do not stay mixed after a period of time. As with initial mixing, the remixing step may be scheduled during a period of inactivity of the robot arm and an aspirator.

When a slide tray 15 is loaded into the apparatus, each bracket 44 is engaging the  
20 locator arm 3 of each cover 2 in the slide tray 15. If an open fill is required, ie where the cover 2 is substantially or fully withdrawn from the slide 1, the locator engaging means 43 moves all covers 2 on the slide tray 15 off the slides to a position such as that shown by cover 2 in figure 8(a). This open position of the cover 2 exposes the sample 5, whereupon the pipette 28 may be positioned in a variety of positions. The  
25 positions of the pipette 28 include either over the sample 5, to dispense fluid directly onto the pipette 28, or adjacent the front of the cover 2 into a fluid reservoir 19 shown in figure 8. The reasons for each position will be explained below.

In an open fill situation, once the fluid has been dispensed on all slides, the locator engaging means 43 moves to position the reaction chambers 32 over the samples on  
30 the slides. Capillary action and the movement of the cover 2 over the surface of the

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slide 1 causes dispensed fluid to flow into the region between the cover 2 and slide 1. The clamp 60 may be used to hold the cover 2 in place and prevent it from floating on the film of liquid between the cover 2 and slide 1.

When the slide 1 is on the block 40, it may be in contact with wicking posts 42, as shown in figures 14 and 15. Movement of the slide 1 on the block 40 is possible as slide lengths vary, and movement of the cover 2 over the slide can move the slide 1. Normally this movement is only in the order of 1-2mm. In another example (not shown) it is possible to use an actuator to move the slide away from the wicking posts to reduce wicking of fluid from the reaction chamber.

Figure 15 shows the cover 2 on the slide 1, both located on block 40. The wicking posts 42 are in contact with the slide and therefore provide a wicking path for fluid. The reaction chamber is located between the slide and cover but as figure 15 is approximately to scale, it cannot be clearly seen in this view. Fluid entered in fluid reservoir 19 flows into the reaction chamber and may flow from the reaction chamber down drain 55 associated with the wicking posts 42. To assist in fluid clearance, the air pressure around the wicking posts may be lowered by withdrawing air from the drain 55 by a pump such as a fan (not shown). This will promote fluid flow through the reaction chamber and out the drain 55 if required. Withdrawing the cover from the slide will also promote fluid flow down the drain 55.

The wicking posts will wick fluid even if not touching the slide, as the meniscus of the fluid will extend out from the edge of the slide near the wicking posts if there is fluid pressure from the wicking posts, or if the air pressure in that region is reduced.

The wicking action may, however, be interrupted if required, such as during an incubation period, by manipulating the locator arm 3 so as to move the cover 2 away from the wicking posts 42 a distance sufficient to prevent any further drain of fluid from the reaction chamber.

When dispensed fluid fills the reaction chamber 32 there may be fluid contact between the fluid in the reaction chamber and the wicking posts 42. The upper surfaces of the blocks 40 are at angles approximately 5 degrees to the horizontal with

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the end of the slide adjacent the wicking posts lower than the bar code end of the slide. The angle promotes fluid flow towards the wicking posts 42, which provide the only contact with the slide 1 apart from the block 40. As the wicking posts 42 contact the slide 1 at or near the upper surface of the slide 1, at the lowest end of the slides  
5 upper surface, the fluid will tend to wick from the area in the reaction chamber on the slide adjacent the wicking posts 42 and not from other areas, as there are no other wicking points.

It is possible to control the dispenser 26 to dispense fluid onto the slide in various positions. The fluid may be dispensed towards the bar coded end of the slide, or  
10 towards the wicking post end of the slide if the cover is in an open position. It is also possible for the dispenser to dispense in a "staggered waterfall" arrangement where fluid is dispensed in a number of positions up the slide. The cover may close as the dispenser moves up the slide.

Fluid is dispensed onto the slide 1 in controlled volumes. It has been found that in the  
15 current arrangement, fluid does not wick from the reaction chamber 32 down the wicking posts 42 unless one of two conditions are met. Firstly, there needs to be fluid in the reservoir 19 to push fluid through the reaction chamber 32. The additional fluid displaces the antecedent fluid, which is removed from the reaction chamber. The antecedent fluid is removed from the reaction chamber via the wicking posts. Thus it  
20 is possible to replace a fluid in the reaction chamber by placing fluid in the fluid reservoir. Secondly, a pump can produce a reduced atmospheric pressure around the wicking posts to cause the pressure differential to draw fluid from the reaction chamber. The reaction chamber may also be drained by reducing air pressure around the wicking posts.

25 If no new fluid is to be added to the reaction chamber it is possible to drain the reaction chamber by opening the reaction chamber. This is accomplished by sliding the cover along the slide 1 until the sample is uncovered. The fluid in the reaction chamber will tend to follow the cover off the sample, draining the fluid via the wicking posts. Alternatively, it is possible to turn on the fan to draw fluid from the

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reaction chamber, where the cover can remain in a closed position. A combination of the above is possible.

In some cases, such as where the fluid being applied or in the reaction chamber is particularly viscous, it may be necessary to utilise the pump and apply fluid to the reservoir to cause fluid flow through the reaction chamber. In this way it is possible to change over fluid a controlled way.

The cover 2 and slide 1 are removed from the apparatus 10 when the reaction is complete and therefore the reaction chamber 32 is unique to each reaction. This eliminates the necessity to thoroughly clean a static reaction chamber as required in other apparatus. Further, the reaction chamber is substantially sealed to the environment reducing evaporation and the possibility of the sample drying out.

As the reaction chamber is formed from a slide and a replaceable cover, it is relatively inexpensive to form a reaction chamber, and a new, clean reaction chamber is formed for each reaction, reducing cleaning costs and time, as well as eliminating the possibility of cross contamination with previous reactions or cleaning fluids.

The initial fill with the cover withdrawn (open fill) provides a method of filling the reaction chamber while minimising the formation of voids or bubbles inside the chamber. Due to the reaction chamber having a depth of approximately 100 microns, once the cover is over the slide forming the reaction chamber, it is difficult to flush the chamber of bubbles or voids. Some of the fluids used in the reactions are extremely expensive and may be hazardous, and therefore it is desirable to keep their consumption to a minimum.

A suitable initial fill fluid has been found to be a mixture of water and 25 to 30% glycerol. Small amounts of glycerol do assist in reducing the incidence of bubble formation, as do larger amounts, however it has been found that in some circumstances 25% glycerol by volume works well. Additives such as detergents (Tween for example) may be included to reduce surface tension, which also have proved beneficial in removing voids in some circumstances.

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The use of glycerol reduces the propensity of the fluid to wick from the surface of the slide via extraneous wicking paths. This reduces the number of large voids that form during an initial fill.

To assist in removing any voids that may reside in the reaction chamber after an initial  
5 fill, it has been found that a fluid having reduced surface tension and viscosity, but miscible with water, such as an alcohol like isopropanol, is useful as a flushing fluid.

Typically flushing occurs after a heating phase, as increasing the temperature in the reaction chamber can cause bubbles or voids to form. The use of a low viscosity fluid such as isopropanol can assist in moving the bubbles or voids.

10 Once the reaction chamber is filled with fluid, it is possible to add further fluid without entrapping additional air. Thus, it is possible to change fluids by merely topping up the fluid reservoir, and in some instances, reducing air pressure near the wicking posts. The reaction chamber thus formed exhibits some desirable flow characteristics, in that a new fluid will not tend to mix with the fluid it is replacing.

15 The capillary nature of the reaction chamber does not allow significant turbulent mixing and therefore it is possible to accurately time the changing of fluids without requiring extensive flushing of the chamber or slide surfaces. This allows the start and finish of a reaction to be determined with sufficient accuracy across a range of reactions and fluids.

20 The speed of the cover movement and pressure reduction can effect the volume of residual fluids left behind.

In order to promote reactions in the reaction chamber on the sample, it is possible to move the cover vertically (in the z axis direction as shown in figure 8) on the slide by modulating the load on the clamp 60. The vertical movement assists in mixing the  
25 fluid in a vertical direction as well as a direction across the slide (y-axis direction), rather than along its length. Filling and draining the reaction chamber move fluid along the length of the slide (x-axis direction) and this may be assisted by moving the cover along the x-axis of the slide by moving the arm 44. The blocks 40 may be heated to promote the reaction.

It is desirable in many reactions, for example involving in-situ hybridisation, epitope retrieval, or dewaxing, to heat the fluid in the reaction chamber to a temperature approaching 100 degrees Celsius. In this situation, gas bubbles have been known to form, and the gas bubbles can be difficult to shift. If the bubbles occur on the sample they reduce the amount of fluid exposed to the sample, and can therefore effect the consistency of the result within a sample, as well as between samples on different slides. In such situations it has been found that using covers having one or more coatings can reduce the incidence of bubble formation.

Another feature of the reaction apparatus 10 is that the size of the reaction chamber may be varied. Typically the volume of the reaction chamber when the cover is completely over the slide, termed the closed position, is 150 microlitres. However, if the cover is not completely closed then the reaction chamber formed between the cover and slide may be of reduced volume. In figure 8(b) a cover in a partially closed position is shown, wherein the volume of the reaction chamber would be significantly reduces, for example to 80 microlitres. This example may be useful where samples are small, or placed towards an end of the slid that allows the cover to form a smaller reaction chamber while still covering the sample. Smaller reaction chambers require smaller volumes of fluids, which is advantageous if the fluids used are expensive or difficult to obtain. The examples of the reaction apparatus allow the position of the cover to be referenced when dispensing fluid onto the slide. Therefore, when the cover is in the open position, it is possible to dispense fluid either on top of the tissue sample, or between the tissue sample and the cover, so that movement of the cover to a closed position pushes fluid across the sample while filling the reaction chamber. It is also possible to dispense fluid at a number of positions along the slide, or to dispense fluid on or near the front edge of the cover.

The following is a description of set up and use of the above-described apparatus.

**1. Slide loading:** Paraffin-embedded tissue sections (sample 5) mounted onto glass slides are loaded into the slide tray 15 with covers 2 and inserted into the receiving zones 14 of the reaction apparatus 10. The user selects desired protocols, run type [ie 100  $\mu$ L (economy -2/3 of slide) or 150  $\mu$ L (standard -full slide)] and ensures that the

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reagents trays 34 containing the necessary reagent containers 39 are loaded into the apparatus 10.

2. **Dewaxing:** Removal of wax from tissue sections following sectioning is required prior to performing staining procedures. For dewaxing on the instrument the cover remains in a closed position while dewaxing solution is dispensed by the dispensing means 26 onto the slides, which are pre-heated to 70°C by mounting blocks 40. Slides are incubated for 4 min at 70°C prior to removal of excess dewaxing solution by reduced air pressure around the wicking posts caused by a pump (not shown). Fresh dewaxing solution is dispensed onto the slides for incubation at 70°C for a further 4 min. This process is typically repeated once more for all slides in a tray that require dewaxing. Slides are cooled to ambient temperature and covers opened and closed to remove excess dewaxing solution containing residual dissolved wax. All slides are washed with isopropanol applied by the dispensing means one slide at a time, to remove remaining dewaxing solution, and then all slides are rehydrated with distilled water dispensed by the dispensing means.

3. **Epitope retrieval:** Before IHC and ISH processing can take place, it is necessary to expose epitopes (proteins, DNA, RNA) within the tissue which may have become hidden during the fixation process. On the instrument two protocols may be present:

a. **Heat-induced Epitope Retrieval (HIER)**

Following dewaxing, all slides receive an initial fill of retrieval buffer (initial fill fluid) (10mM Sodium Citrate/30% Glycerol/0.05% Tween) with the cover in the open position to facilitate movement of solution down the slide and reduce bubble formation. Covers are closed and mounting blocks 40 heat the slides to 100°C for the required retrieval time. After retrieval is finished, slides are cooled by individual flushing with retrieval buffer by the dispensing means.



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**b. Enzyme-induced Epitope Retrieval (EIER)**

Protease solution (ie proteinase K, pepsin, and trypsin) is dispensed onto each slide by the dispensing means and incubated for 10-30 minutes at the desired retrieval temperature (for example ambient-50°C or room temperature). After  
5 retrieval is complete, each slide is washed with distilled water dispensed by the dispensing means.

**4. Immunohistochemistry (IHC):** IHC is based on specific binding of antibodies (proteins) to antigens (proteins) in tissue biopsies and specimens. Following the epitope retrieval stage, each slide receives buffer containing Tween-20 from the  
10 dispensing means. Each slide may be treated with hydrogen peroxide for 8 min at ambient temperature to block endogenous peroxidase activity within the tissue sections and is washed with TWB buffer containing Tween-20, again dispensed by the dispensing means. A primary antibody directed against a specific target protein is applied by the dispensing means to the tissue sample and incubated for 15-60 min.  
15 This is followed by a secondary biotin-labelled antibody incubation. Bound antibody is detected by dispensing streptavidin- or alkaline phosphatase-conjugated peroxidase onto each slide, which is visualised by addition of a chromogen (ie DAB, BCIP/NBT), all by dispensed by the dispensing means. Sections are counterstained with hematoxylin, also dispensed by the dispensing means..

**5. In situ hybridisation (ISH):** ISH allows the detection of specific nucleic acid sequences within a cell. Following the EIER stage, tissue sections are dehydrated by dispensing isopropanol into the reaction chambers of each slide and the cover moved to the open position to dry the tissue. A fluorescein- or biotin-labelled nucleic acid probe is applied to the slide and the cover closed slowly to distribute the probe evenly  
25 across the tissue. The probe is allowed to hybridise to its complementary DNA/RNA target in a tissue section for 1.5-2 hours at 37-55°C. Where the target is DNA, the tissue section and probe are first denatured at high temperature (ie 95°C) for 5-10 min prior to hybridisation. Slides are washed by dispensing TWB from the dispensing means using a staggered waterfall rinse to gently remove unbound probe. Following  
30 washing, the cover is moved to the closed position for the remainder of the procedure.

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Bound probe is detected by applying an anti-fluorescein or anti-biotin antibody conjugated to alkaline phosphatase, dispensed from the dispensing means, which is visualised by addition of an enzyme substrate (BCIP/NBT), also dispensed from the dispensing means.

- 5     **6. Removal:** Once the protocol has been completed for a particular slide tray, the tray may be removed regardless of the status of the other slide trays. As the slide tray may contain slides each having different protocols applied, the tray must remain in the apparatus until all protocols for that particular tray have been completed. An indicator such as a light informs the user when all the protocols to be applied to the slides on  
10    the slide tray have been completed.

- Once the reaction chamber has been filled it is possible to hold the sample in a buffer for an extended period of time. Fluid in the reaction chamber can be topped up if, for example, some slides reactions are completed but other slides on a slide tray require additional processing. Having three slide trays allows a certain amount of flexibility  
15    in that samples that require time intensive processing can be placed in one slide tray, while faster processing may be undertaken on a separate slide tray. An additional slide tray may be entered while one or more slide trays have begun processing, and it is possible to remove a finished slide tray while another slide tray is being processed. The reagent racks 34 may be removed during a process run, if for example, a  
20    container empties. Once the reagent rack 34 is replaced, the bar code sensor 33 scans the bar codes on the reagent containers again to ensure that only the correct reagents are applied.

- The dispensing mechanism employs a sensor to detect the level of the fluid in the reagent container, and therefore warns the user when the container is running low.  
25    This is important as reagent may have a short useful life when not stored properly, and the reagent is also expensive, therefore there are significant advantages in reducing waste.

- The sensor may be attached to the pipette to sense when the pipette reaches the surface of the fluid in the reagent container. This allows the volume of a container to  
30    be determined, and a warning maybe sent to the operator is fluid levels drop to a

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predetermined level. The reagent rack may then be removed from the apparatus, the container replaced, whereupon the scanner will determine whether the correct reagent was replaced by reading the bar code on the reagent container. In this way operator error is reduced.

- 5     There are a number of variations described herein, but the apparatus is designed to allow a flexible approach to fluid application, reaction time and temperature. It is therefore not intended that the apparatus be limited to particular examples of potential methodology, as variations in fluid application, cover position and movement.

- 10    The protocols that may be applied are varied, and it is possible to apply a different protocol to each sample on a slide in a single rack. Further, it is possible to load a new tray of slides or remove a completed tray of slides while the apparatus is processing another tray of slides.

Without limiting the forgoing, some specific aspects of the invention are recited below, together with a brief description of some advantages of each:

- 15    A method of forming a reaction chamber on a slide in a reaction apparatus including:  
placing a cover having a cavity on a slide, forming a reaction chamber;  
locating the cover and slide in a receptacle of a tray;  
providing a receiving portion in the reaction apparatus having a mount for each receptacle in the tray;  
20    loading the tray into a receiving portion of the reaction apparatus, where the receiving portion of the reaction apparatus locates the tray;  
releasably holding the cover to the slide; and  
releasing the tray from the slide and cover.

- 25    The above-mentioned method allows a slide and cover to be easily placed into receptacles in a tray. The tray may have a number of receptacles, for example 10 receptacles per tray as shown in the figures of the embodiments disclosed herein. The tray can then be loaded into a receiving portion of the reaction apparatus, so that, for example up to 10 reaction chambers formed from slides and covers, can be placed into

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the reaction apparatus. As the tray is located by the reaction apparatus upon loading, and the slides and covers are located by the tray, the exact position of up to 10 reaction chambers can be determined easily within the apparatus. Given that slide dimensions vary due to manufacturing inaccuracies, and the covers do not contact the sides of the slides (to eliminate extraneous wicking points), such that the covers can move freely, on top of the slides if not constrained by other means, locating 10 reaction chambers at once can be difficult.

Once the tray is loaded and the slides and covers are fixed in position by the clamps, the tray can be removed. In the present examples the tray is dropped down so that the mounts support the slides and covers. This removes all contact around the edges of the slides except for the wicking posts. Thus it is possible with this arrangement to easily and quickly locate a number of slides and covers without any contact with the sides of the slides. As the covers do not have a positive sealing arrangement, and the reaction chamber is generally full of fluid, this arrangement assists in loading multiple slides without fluid loss thereby minimising bubble formation within the reaction chamber.

- An apparatus for loading multiple slides and covers including a tray having a number of receptacles for slides and covers;
- a receiving portion for receiving trays;
- mounts for each receptacle located in the receiving portions;
- a clamp for each mount;
- wherein when a tray having slides and covers is loaded into the receiving portion, each clamp holds the cover on the slide to locate the slide, and the tray drops from the slides so each slide is supported by the mount.
- Preferably, a draining means is provided.

Preferably the draining means includes a wicking means.

The apparatus above allows slides and covers to be loaded easily by an operator, in batches if required.

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A method of undertaking reactions on samples on slides involving multiple steps including:

loading a first holder having at least one slide into a reaction apparatus;

scanning the slide to determine the multiple steps in the reaction to take place on the  
5 slide;

determining whether other holders have been loaded into the reaction apparatus;

undertaking the multiple steps required on the at least one slide associated with the first holder;

when the second batch is detected, continue the steps in the reaction associated with  
10 the at least one slides in the first holder and then undertaking the at least one steps associated with the slides associated with the second holder.

This is possible in some situations as there are usually gaps where the apparatus used to start or stop reactions, or undertake other tasks (such as the pipette mounted to the robot arm) are not utilised all the time.

15 If the apparatus are used all the time then the steps of the reaction to take place on the at least one slides associated with the second holder will not commence until the first bath has finished.

An apparatus for performing reactions on slides including a tray having a plurality of receptacles adapted to support and locate slides and associated covers

20 receiving ports for the trays, the receiving ports having mounts associated with each receptacle of the tray;

a clamping mechanism for clamping the cover and slide in place;

a fluid draining means for draining fluid from the reaction chamber formed between the cover and slide;

25 fluid receptacles to allow at least one fluid to be placed on the apparatus

fluid dispensing means to dispense fluid onto the slides

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wherein once the tray is loaded, the slides and cover are clamped and the tray is moved so that the slides and covers are supported on the mounts, fluid may be dispensed onto the slides by the dispensing means, and drained by the draining means.

Preferably, the apparatus includes a locating means for locating and moving the cover  
5 with respect to the slide.

Preferably there is a locating means associated with every receptacle in a tray.

Preferably all locating means associated with a particular tray all move at the same time to move the cover with respect to the slide, to facilitate fluid dispensation or draining of all slides on a tray.

10 An apparatus for applying reagents to sample slides, including:

a plurality of ports for receiving the slides;

a reader for reading identification information on each of the slides; and

a reagent rack for receiving reagent containers which carry reagent to be deposited on the slides; wherein

15 the slides are provided on trays, which are received in the associated ports such that each tray represents a separate batch of slides, to allow for addition and removal of separate trays, for batch processing during operation of the apparatus.

The batch loading, again, provides substantial flexibility for an operator insofar as testing and scheduling is concerned.

20